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Mammographic breast density, has been shown to be a major risk factor for breast. We hypothesize that there is at least one major gene involved in the genetic variation of breast density. We propose to identify the chromosomal location for this gene(s) by linkage analysis. To do so, we have selected *23* large families providing the greatest information for linkage from a total of 426 breast cancer families in the Minnesota Breast Cancer Family Study. Mammograms and risk factor data were previously collected on these women through interview and mailed questionnaires. As of October 30, 2000, 384 samples from these families have been collected for analysis. We have also concentrated efforts on improving the breast density estimate. We have updated the subjective estimate of breast density to a computer-assisted estimate. Additionally, in the past year, we have performed several small studies to evaluate the reliability of this computer-estimate of density. The final year of the study will involve a genome screen for loci linked to the breast density trait, using a screening set of microsatellite markers that span the genome. The discovery of breast density genes could help in the identification of susceptible individuals to target prevention strategies.

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Table of Contents

Introduction

The interindividual variability in breast tissue on mammographic images, as defined by several measures of mammographic breast density, has been shown to be a major risk factor for breast cancer with three to five-fold increases in risk associated with densities greater than 50% (Boyd, 1998). We have previously demonstrated a genetic component to the mammographic breast density trait (Pankow, 1997). Our hypothesis is that there is at least one major gene involved in the genetic variation of breast density, but there are no obvious candidate genes or chromosomal regions known with certainty. We now propose to identify the chromosomal location for this gene(s) by linkage analysis, as the first step in identifying the gene(s) that is responsible for the differences in breast density. In the body of this report, we will describe the progress to date on the search for the breast density gene(s). The discovery of breast density genes could help in the identification of susceptible individuals to target prevention strategies as well as provide insight into the development of breast cancer.

Body

The goal of our study is to locate a chromosomal region for a gene for breast density. The major emphasis of this first phase of this linkage study has been to select informative families for linkage and collect DNA and trait information on these family members. We selected 23 families for linkage, based on simulation analyses that incorporated the trait information. We collected 384 blood samples on eligible women in these families who had mammogram and risk factor information. Our participation rate for the blood component of the study was 79%. The distribution of bloods per family is described in the table below.

Table 1. Distribution of bloods for 23 families in linkage analysis

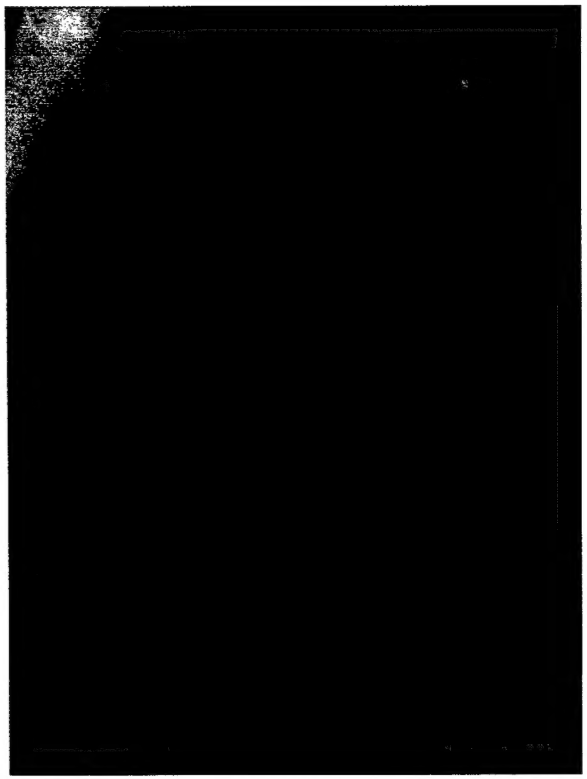
Family No.	No. Bloods
018	11
042	9
055	15
086	16
115	29
165	39
198	8
263	10
285	22
316	14
400	14
406	16
418	15
433	8
438	31
464	12
498	17
504	10
506	19
540	16
549	27
588	18
589	8

The blood collection phase is now complete. As a result, the focus of the study will now shift to genotyping a panel of microsatellite markers for these 384 individuals. The panel consists of a screening set of microsatellite markers that are distributed across all autosomes and separated at an average distance of 10 centimorgans (ABI marker set). We approximate 6-8 months to complete the genotyping of these 23 families.

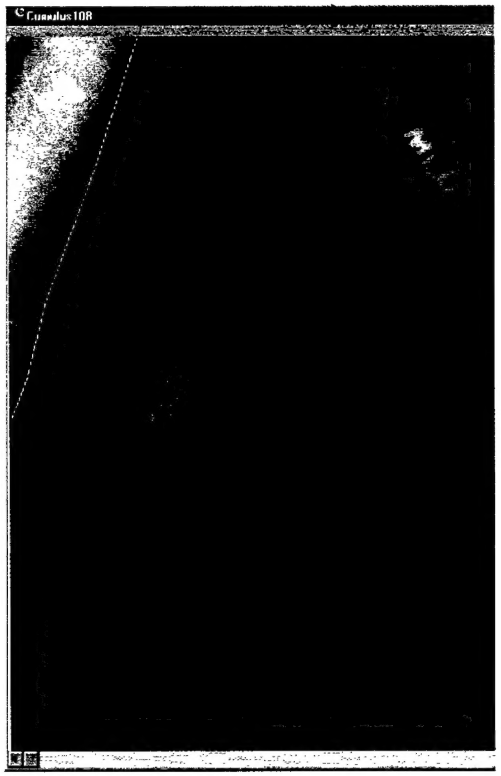
Also integral to any linkage analysis is a clearly defined trait. In our case, the trait of interest is mammographic breast density, which is defined as the percent of the breast that is composed of nonfatty tissue. However, there are several ways in which to measure percent density. Previously, in our segregation analysis, we used a subjective estimate by an experienced radiologist, which was shown to have high reliability and validity (compared to an MRI measure of the breast). However, we desired a more precise measure and have acquired software for a computer-assisted estimate of breast density devloped by Martin Yaffe and colleagues at the University of Toronto (Yaffe, 1994). The procedure for setting thresholds on the mammographic image to arrive at a density estimate is pictorially described in Figure 1.

In the past year, we have performed studies to evaluate the computer-assisted estimate. We have trained a programmer to apply the algorithm and continually compute her intra and inter reliability estimates (comparing her estimates to those of Dr. Norman Boyd at the University of Toronto, an expert in breast density estimation). Her intra reader reliability is consistently above 90%, and her inter reader reliability with Dr. Norman Boyd on a set of 100 digitized images was 95%. Once the programmer has completed reading the mammograms for this study, we will compute the correlation between the subjective estimate of density and the computer-assisted estimate. If the correlation exceeds 90% then, we will perform our linkage analyses using the computer-assisted estimate of breast density rather than the subjective estimate.

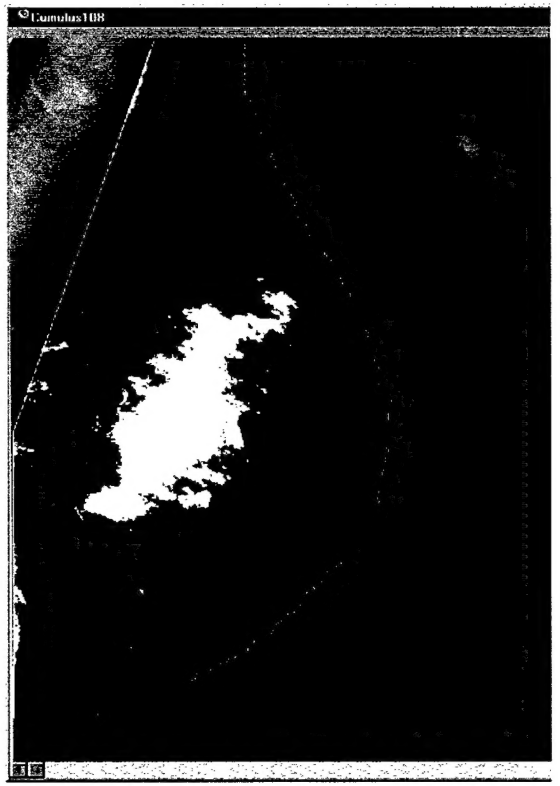
Figure 1: Example of Percent Density Estimation



Digitized mammogram



Setting thresholds



Dense area highlighted

Key Research Accomplishments

- Performed simulation analyses under nonparametric and paramteric linkage to determine the most informative families for linkage analysis.
- Collected blood from 384 family members in 23 families with trait and risk factor information. Participation rate was 79%.
- Implemented a computer-assisted algorithm for estimation of percent mammographic breast density.
- Performing a comparison of the estimates from computer-assisted method with subjective estimates of density.
- Performed intra and inter reliability studies to evaluate the performance of the computer-assisted method.
- Currently are preparing to begin genotyping for the genome screen. Estimated completion time will be 6-8 months.

Reportable Outcomes

Work as of 10/2000 has involved blood collection and DNA extraction for 384 participants. The only reportable outcome was our poster presentation at the DOD meeting last June.

Vachon CM, Thibodeau SN, Kulby VJ, Sellers TA. Genetic linkage analysis of mammographic breast density. Department of Defense Breast Cancer Research Program Meeting, June 2000.

Conclusions

The majority of the past two years has involved the collection of blood samples as sources of DNA for the genome screen. Our participation has been above average, with 79% of women participating in the study. We have also devoted a large effort to developing a more adequate estimate of breast density. Having performed several studies on the performance of the computer-assisted method, we are confident that this measure will be appropriate for our linkage analysis. The final year of the study will involve the genotyping for the 384 women in these families. We will perform both parametric and nonparametric linkage analyses to hopefully arrive at a chromosomal location for a breast density gene.

References

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